

Conclusions. 1. The Wassermann reaction is invariably positive at some time during the course of syphilis, if properly performed, and at sufficiently frequent intervals.

2. It is but rarely positive in non-luetic diseases.

3. It is unusual for it to be positive in malaria. If positive, it is only weakly so (doubtful in Craig's classification) while the fever is rising, becoming negative between paroxysms.

4. It is rarely positive in tuberculosis, and when positive the reaction is weak or doubtful (*i. e.*, not diagnostic of lues in the absence of history or signs). I have found it positive only in far-advanced cases shortly before death.

5. It is not positive in uncomplicated hyperthyroidism.

6. The cholesterinized antigen is probably too delicate to be of value alone in diagnosing lues when there is no history or physical evidence of disease. It is invaluable in following the course of known syphilis under treatment, by reason of its delicacy.

I desire to express here my deep gratitude to Dr. Edward B. Krumbhaar, director of laboratories, Philadelphia General Hospital, for his unfailing kindness and great courtesy to a beginner in medicine.

A FURTHER NOTE UPON A COMPARISON OF THE SACHS-GEORGI AND WASSERMANN REACTIONS IN THE SEROLOGIC DIAGNOSIS OF SYPHILIS.

By ROBERT A. KILDUFFE, M.D.,

DIRECTOR OF LABORATORIES, PITTSBURGH HOSPITAL; DIRECTOR OF LABORATORIES, MCKEESPORT HOSPITAL; SEROLOGIST, PROVIDENCE HOSPITAL.

(From the Laboratories of the Pittsburgh Hospital.)

IN a former communication¹ a report was made of the results obtained with the Sachs-Georgi reaction in a series of 296 serums simultaneously subjected to the Wassermann test.

At that time, as the result of the observations made and recorded, the conclusion was reached that "A diagnosis of syphilis or conclusions as to treatment cannot be based upon the results of a Sachs-Georgi test with safety and the reaction is not suitable for general use for this purpose."

Since the publication of this report several others appeared both in foreign and American literature. The foreign reports were almost uniformly favorable, while those of American literature, though varying to some extent, were, in the main, corroborative

¹ Kilduffe, R. A.: A Comparison of the Wassermann and Sachs-Georgi Reaction in the Serologic Diagnosis of Syphilis, *Arch. Dermat. and Syph.*, April, 1921, 3, 1, 415.

of the usefulness of the test. Notable among these are the papers of Hull and Faught² and Levinson and Peterson.³

The former observers, using a modification of the test in which the test serum is used undiluted in amounts of 0.3 cc, found that their modified reaction agreed with the Wassermann in 88 per cent of 296 serums tested by both methods.

Levinson and Peterson, reporting upon 1042 comparative tests using the modification proposed by Mandelbaum,⁴ in which the serum is diluted in the proportion of 3 drops to 1 cc of normal saline solution, with the incubation period in the hot-air incubator lengthened to eighteen to twenty-four hours, followed by twenty-four hours at room temperature, found that the two reactions agreed in 92 per cent of the cases tested.

Immediately following the conclusion of the first series published from these laboratories, and in view of the varying results recorded elsewhere, it was thought that perhaps the antigen used had not sufficient delicacy, and a new antigen was thereupon prepared and a new series inaugurated, the results of which are herewith reported.

In the preparation of the antigen the technic as given by Galli-Valerie⁵ was adhered to, as it seems to be that used by all investigators publishing their technic. Absolute alcohol was used throughout and Merck's cholesterin for the cholesterinization of the alcoholic extract. Before cholesterinization the alcoholic beef heart was titrated and found to be anticomplementary in 2.5 cc of 1 to 20 dilution and antigenic in 0.04 cc of 1 to 20 dilution. After the addition of the further 200 cc of alcohol and the 1 per cent alcoholic cholesterin solution (13.5 cc) the anticomplementary titration remained the same while the antigenic unit had fallen to 0.1 cc of 1 to 20 dilution, probably because of the further dilution of the extract by the added alcohol. This antigen was then added to a series of known positive and negative serums in order to determine that precipitation would occur in a sufficient number to indicate its availability for use in the test.

A series of 430 serums was then subjected to parallel Wassermann and Sachs-Georgi tests by the following technic:

Technic of the Tests. Sachs-Georgi: The antigen prepared and titrated as indicated above was diluted 1 to 5 with sterile salt solution (0.85 per cent), one part of the saline being added slowly with gentle shaking and the remaining four parts added more rapidly, the shaking being continued. The resultant solution was turbid and milky.

² The Sachs-Georgi Precipitation Test for Syphilis, *Jour. Immunol.*, November, 1920, 6, 521.

³ The Sachs-Georgi Reaction for Syphilis, *Arch. Dermat. and Syph.*, March, 1921, 3, 286.

⁴ *München. med. Wehnschr.*, 1918, 65, 204, quoted by Levinson and Peterson.

⁵ *Corr.-Bl. f. schweiz. Aerzte*, December 25, 1919, 49, 1978.

Serums: The serums used in the series comprised those taken for diagnosis in the wards of the Pittsburgh Hospital; serums taken as a routine in the gynecologic and obstetric services of the hospital, and those of cases handled through the Morals Court, these last being obtained through the coöperation of Dr. A. H. Eggers, County Medical Inspector, Pennsylvania State Department of Health. In this latter group were known syphilitics, suspects and cases under treatment. A sufficient number of normal Wassermann-negative serums were included in the series to serve as an adequate control.

No serum used was over forty-eight hours old, and many were tested within twenty-four hours of their receipt. All were clear, free from blood and inactivated for fifteen minutes at 56° C.

For the test 0.1 cc of serum was placed in a serologic test-tube and 0.9 cc of sterile 0.85 per cent salt solution added, thus giving 1 cc of a 1 to 10 dilution, to which 0.5 cc of the Sachs-Georgi antigen was added and the tubes gently shaken. They were then placed in the hot-air incubator for eighteen to twenty-four hours, a preliminary reading made and then allowed to stand at room temperature for twenty-four hours, after which the final reading was made. A known negative and positive were always included. Readings were made by transmitted light, using a modified agglutinoscope and recorded as follows:

- ++++ Well-marked precipitate plainly discernible.
- +++ Perceptible precipitate not so marked as ++++.
- ++ Perceptible precipitate not so heavy as +++.
- ± Barely discernible trace of precipitate.
- No precipitate.

Technic of the Wassermann Test. All serums were submitted to the Wassermann test at the same time by the following technic:

Sheep Cells: Sheep bled the day preceding the test, cells washed until free from serum (four to six washings in normal saline) and used in dose of 0.5 cc of a 2.5 per cent suspension.

Amboceptor: Glycerinated serum diluted with normal saline according to titer and used in dose of 0.2 cc.

Complement: The pooled serum of two to four guinea pigs diluted 1 to 10 with 0.85 per cent salt solution. Each day preceding the main test, complement was titrated against 0.5 cc of 2.5 per cent sheep-cell suspension in the presence of 0.1 cc of amboceptor and the unit read as the smallest amount producing complete hemolysis in one hour in the 38° C. water-bath. Two units were used in the test.

Antigens: A triple battery was used for each serum, consisting of cholesterinized (0.4 per cent) extract of human heart; acetone-insoluble lipoids of human heart; and an alcoholic extract of syphilitic fetal liver. The dose was determined by antigenic and anticomplementary titrations, the amount used being always from two to five times the antigenic unit, which amount was always from five to ten times less than the anticomplementary unit.

The Test: Each serum was used in dose of 0.2 cc and the complement in dose of two titrated units of 1 to 10 dilution. Incubation was in an iced water-bath at a temperature of 2° to 4° C. for one hour. At the end of the preliminary incubation the tubes were placed in the 38° C. bath for five minutes and then the cells and amboceptor added and the test reincubated in the 38° C. bath until all controls were hemolyzed.

Antigen, corpuscle, amboceptor, complement and positive and negative controls were always added. The same antigens were used throughout the tests.

Results were recorded according to the degree of fixation obtained with each antigen separately and the strength of the reaction graded accordingly. A serum was looked upon as positive if 50 per cent or over of inhibition of hemolysis occurred with the cholesterol antigen alone, even though the other two antigens were negative as, on the basis of data elsewhere recorded,⁶ and as said by Kolmer,⁷ "I have learned from experience to place reliance upon results obtained with a properly prepared and titrated cholesterolized extract."

Results of Comparison of the two Reactions. A total of 430 cases was subjected to parallel tests as above outlined. Of these 102, or approximately 23 per cent, gave plus-four fixations, the high percentage of positive cases being largely due to the inclusion in the series of the Morals Court serums, in which a high percentage of positive findings was to be expected, which, for the purpose of this investigation, was to be desired.

Of the Wassermann positive cases, as shown in Table I, the Sachs-Georgi was positive in 30 and negative in 72.

TABLE I.—RESULTS WITH WASSERMANN POSITIVE SERUMS.*

Number of serums.	Sachs-Georgi reaction.					Sachs-Georgi positive.	Sachs-Georgi negative.
	++++	+++	++	+	—		
102	11	5	10	4	72	30	72

* Includes serums positive with cholesterolized antigens only.

Table II shows the results obtained with 324 Wassermann negative serums in which the Sachs-Georgi reaction gave 32, or approximately 10 per cent of positive results.

⁶ Kilduffe, R. A.: Concerning the Specificity of Cholesterolized Antigens in the Serologic Diagnosis of Syphilis, Arch. Dermat. and Syph., May, 1921, 3, 598.

⁷ The Serum Diagnosis of Syphilis and Gonorrhea Employing Human Complement, Am. Jour. Syph., October, 1918, 2, 739.

TABLE II.—RESULTS WITH WASSERMANN NEGATIVE SERUMS.

Number of serums.	Sachs-Georgi reaction.					Sachs-Georgi positive.	Sachs-Georgi negative.
	++++	+++	++	+	-		
324	6	18	3	5	292	32	292

Four anticomplementary serums were all positive to the Sachs-Georgi test.

TABLE III.—RESULTS WITH ANTICOMPLEMENTARY SERUMS.

Number of serums.	Sachs-Georgi reaction.					Sachs-Georgi positive.	Sachs-Georgi negative.
	++++	+++	++	+	-		
4	2	1	1	0	0	4	0

In the first series similar results were obtained and, for the purpose of consolidation, the combined results of both series are shown in tabular form.

TABLE IV.—COMBINED RESULTS WITH WASSERMANN POSITIVE SERUMS.*

Number of serums.	Sachs-Georgi reaction.					Sachs-Georgi positive.	Sachs-Georgi negative.
	++++	+++	++	+	-		
162	15	5	13	14	115	47	115

* All positive serums, including cholesterin plus only in both series.

TABLE V.—COMBINED RESULTS WITH WASSERMANN NEGATIVE SERUMS.*

Number of serums.	Sachs-Georgi reaction.					Sachs-Georgi positive.	Sachs-Georgi negative.
	++++	+++	++	+	-		
544	11	18	7	18	490	54	490

* Includes serums of both series.

TABLE VI.—COMBINED RESULTS WITH ANTICOMPLEMENTARY SERUMS.*

Number of serums.	Sachs-Georgi reactions.					Sachs-Georgi positive.	Sachs-Georgi negative.
	++++	+++	++	+	—		
20	2	1	1	2	14	6	14

* Includes serums of both series.

A study of the tables shows that of 162 Wassermann positive serums, 47, or approximately 29 per cent, were positive to the Sachs-Georgi reaction as here performed, and that in 544 negative serums the Sachs-Georgi reaction was also positive in approximately 10 per cent.

Of 20 anticomplementary serums, 6, or approximately 30 per cent, gave positive Sachs-Georgi reactions.

Every effort was made throughout the present series to make the tests strictly parallel and to perform the Sachs-Georgi reactions with great care in view of the variance from the results obtained by other investigators. The results are obvious and indicate either that the conclusions previously drawn may be reaffirmed and looked upon as corroborated or that the Sachs-Georgi reaction was improperly performed. The only deviation from the original technic was the substitution of 1 cc of 1 to 10 dilution for 10 cc of the same dilution (1:10), and it is not felt that this had any appreciable influence upon the results obtained.

Summary. The results of 430 parallel Wassermann and Sachs-Georgi reactions are reported and coördinated with a series of 296 previously reported.

Conclusions. The following conclusions, previously stated, are reaffirmed upon the basis of the results herewith reported:

1. The Sachs-Georgi test is often difficult to read.
2. The reaction is neither as delicate nor as trustworthy as the Wassermann test.
3. The Sachs-Georgi reaction may be positive with Wassermann negative serums and negative with Wassermann positive serums in relatively high percentage of cases.
4. The number of non-specific reactions is sufficiently high to render the test unreliable as a means of diagnosis.
5. A diagnosis of syphilis, or conclusions as to the results of treatment, cannot be based upon the results of a Sachs-Georgi reaction with safety, and the reaction does not seem suitable for general use for this purpose.